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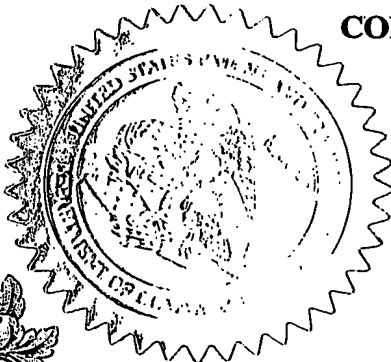
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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

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<input type="checkbox"/> Additional inventors are being named on the _____ separately numbered sheets attached hereto					
TITLE OF THE INVENTION (280 characters max)					
KIT FOR MOLECULAR IDENTIFICATION OF SMALLPOX					
Direct all correspondence to: CORRESPONDENCE ADDRESS					
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ENCLOSED APPLICATION PARTS (check all that apply)					
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METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT (check one)					
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Respectfully submitted,

SIGNATURE

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16/04/03

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REGISTRATION NO.

45,333

(If appropriate)

Docket Number:

85084-572

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This collection of information is required by 37 CFR 1.51. The information is used by the public to file (and by the PTO to process) a provisional application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 8 hours to complete, including gathering, preparing, and submitting the complete provisional application to the PTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, Washington, D.C. 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Box Provisional Application, Assistant Commissioner for Patents, Washington, D.C.

P18LARGE/REV05

KIT FOR MOLECULAR IDENTIFICATION OF SMALLPOX

FIELD OF THE INVENTION

The present invention relates generally to the field of pathogen identification.

5

BACKGROUND OF THE INVENTION

Currently, international concern is heightened regarding the use of Smallpox virus as a bioterrorism agent. Smallpox is a disease caused by an infection with the variola virus, a member of the genus Orthopoxvirus. The last naturally occurring case
10 of Smallpox was reported in Somalia in 1977. Since recommendations for routine Smallpox vaccination were rescinded in North America and most of Europe in 1971, and the effectiveness of vaccination appears to last only 10 years, much of the world population is currently susceptible to infection. During the Smallpox era, overall mortality rates were approximately 30%. Death usually occurred late in the first week
15 or during the second week of illness and was usually attributed to overwhelming viremia. The virus is highly transmissible from person-to-person and infected individuals may, in turn, infect tens to hundreds of susceptible contacts.

The only acknowledged stockpiles of variola virus, the causative agent of smallpox, are those maintained in the USA and Russia (Henderson, D.A. et al. JAMA
20 281,2127-2137 (1999)). The recent anthrax attack on the USA, however, has renewed fears that additional stockpiles do exist and could be used as a bioterrorist weapon on a now largely susceptible population. The diagnosis of ordinary-type smallpox was relatively easy when endemic, and was based on the distribution and

evolution of the rash. However, in non-endemic regions, smallpox could sometimes be confused with chickenpox which is caused by a herpesvirus (varicella) (Fields' virology, Knipe, D.M., Howley, P.M. (eds)-4th ed., 2001). Monkeypox virus produces a clinically indistinguishable disease, with the exception of enlargement of cervical and inguinal lymph nodes; outbreaks thus far have been limited to tropical rainforest regions of Central and Western Africa (Fields' virology, Knipe, D.M., Howley, P.M. (eds)-4th ed., 2001).

Thus, as discussed above, when faced with a potential smallpox or monkeypox outbreak, time is of the essence. Clearly, a quick and easy method of determining if orthopoxvirus is present within a sample and identifying the orthopoxvirus is needed.

SUMMARY OF THE INVENTION

According to a first aspect of the invention, there is provided a method of detecting and identifying an orthopoxvirus within a sample comprising:

adding to the sample reagents for nucleic acid amplification and at least one pair of primers capable of amplifying at least one region of the orthopoxvirus genome, said region of the orthopoxvirus genome selected from the group consisting of HA and crmB;

incubating the sample under conditions suitable for nucleic acid amplification thereby producing an amplicon if the sample contains orthopoxvirus;

adding at least one restriction enzyme selected from the group consisting of: Sau 3AI, Spe I, Dra I, Hpa I, Ssp I, Alw 44I and combinations thereof; and

determining if restriction enzyme digestion of an amplicon has occurred.

According to a second aspect of the invention, there is provided a pair of primers for detecting orthopoxvirus in a sample comprising 12 or more consecutive nucleotides of ATGCCGGTACTTATGTATGTGC (SPOXHA5, SEQ ID NO: 1) and 12 or more consecutive nucleotides of TCTTGTCTGTTGTGGATTCT (SPOXHA3, SEQ ID NO: 2) or 12 or more consecutive nucleotides of TACCGGTCTCAGCGAATC (SPOXcrmB5, SEQ ID NO: 3) and 12 or more consecutive nucleotides of ACCGTCTCCGAATGCGGCAT (SPOXcrmB3, SEQ ID NO: 4).

According to a third aspect of the invention, there is provided a kit for detecting and identifying orthopoxvirus comprising:

- 10 at least one pair of primers selected from the group consisting of 12 or more consecutive nucleotides of ATGCCGGTACTTATGTATGTGC (SPOXHA5, SEQ ID NO: 1) and 12 or more consecutive nucleotides of TCTTGTCTGTTGTGGATTCT (SPOXHA3, SEQ ID NO: 2); and 12 or more consecutive nucleotides of TACCGGTCTCAGCGAATC (SPOXcrmB5, SEQ ID NO: 3) and 12 or more consecutive nucleotides of ACCGTCTCCGAATGCGGCAT (SPOXcrmB3, SEQ ID NO: 4).

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1: Schematic representation of restriction enzyme sites used for RFLP analysis of Orthopoxvirus PCR generated amplicons. Open bars represent the amplicons produced by the HA and crmB primer sets designed for this study. a, Sequence of primers for HA amplification corresponded to base pairs 151327-151347 (SpoxHA5) and 151656-151637 (SpoxHA3) of variola major strain (Bangladesh 1975)

GenBank accession # 1-22579. Amplicons generated using these primers were used in RFLP analysis using Sau 3AI (black arrows) and Spe I (open arrows). These two enzymes were sufficient to differentiate variola, monkeypox and camelpox from each other as well as from vaccinia or cowpox which produce identical fragments in this assay. **b**, Sequence of primers for crmB amplification corresponded to base pairs 183227-183244 (SpoxcrmB5) and 183493-183474 (SpoxcrmB3) of variola major strain (Bangladesh 1975). Amplicons generated using these primers were used in RFLP analysis with Dra I (black arrow), Hpa I (open arrow), Ssp I (grey arrow) and Alw44 I (diagonally striped arrow). Variola sequence contains a Dra I site that is unique to itself allowing its differentiation. Cowpox and vaccinia are both unique in containing an Alw44 I site differentiating these two from the other species. Amplicons negative for both Dra I and Alw44 I can be identified as monkeypox upon digestion with Hpa I or camelpox if digestion occurs with Ssp I. Approximate size of RFLP generated fragments are shown in the table to the right.

15

Figure 2: RFLP analysis of amplicons produced by PCR using SpoxHA and SpoxcrmB primer sets. Restriction fragments were separated on a 8% PAGE gel and visualized with ethidium bromide. **a**, Digestion of SpoxHA amplicons produced from positive controls for variola, monkeypox, camelpox and vaccinia with Sau 3AI and Spe I show the expected bands from sequence analysis. Test sample (MVA T7) produced banding pattern consistent only with vaccinia and cowpox genotype. **b**, Digestion of SpoxcrmB amplicons produced from positive controls for variola, monkeypox, camelpox and vaccinia show expected bands from sequence analysis. Dra I does not

20

digest the test amplicon, eliminating variola from the differential diagnosis. Hpa I sites are present only in vaccinia and cowpox (not shown) or monkeypox limiting the possible identity of the test sample to only these viruses. Lack of digestion by Ssp I confirms that camelpox is not a possible identity. Alw44 I does digest the test
5 amplicon resulting in a putative diagnosis of vaccinia/cowpox infection.


DESCRIPTION OF THE PREFERRED EMBODIMENTS

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which
10 the invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are now described. All publications mentioned hereunder are incorporated herein by reference.

15 DEFINITIONS

As used herein, "amplification reaction mixture" or "amplification mixture" refers to an aqueous solution comprising the various reagents used to amplify a target nucleic acid. These include but are by no means limited to enzymes, aqueous buffers, salts, target nucleic acid and nucleoside triphosphates.

20 As used herein, "isolated" or "substantially pure", when referring to nucleic acids, refers to those which have been purified away from other cellular components and/or contaminants by standard techniques, for example, column chromatography, CsCl banding, and alkaline/SDS treatment as well as other techniques well known in



the art.

As used herein, "nucleotide polymerase" refers to enzymes that are capable of catalyzing the synthesis of DNA or RNA from nucleoside triphosphate precursors.


As used herein, "primer" refers to an oligonucleotide capable of acting as a
5 point of initiation of DNA synthesis under conditions in which synthesis of a primer extension product complementary to a nucleic acid strand is initiated.

As used herein, "orthopoxvirus" refers to for example but by no means limited to variola, monkeypox, camelpox, vaccinia and/or cowpox viruses.

Proper annealing conditions depend, for example, on the length of the primer
10 or probe, the base composition of said primer or probe and the number of mismatches present and their relative position.

Variola virus is considered an "A" list biological agent of high bioweapon capability and as such, rapid, sensitive and controlled diagnostic assays are needed to confirm any putative clinical cases. We developed two polymerase chain reaction -
15 restriction fragment length polymorphism (PCR-RFLP) based assays to detect variola virus and differentiate it from the clinically similar monkeypox virus and from the related vaccinia and camelpox viruses, utilizing artificially generated RFLP positive controls in place of the unavailable variola and monkeypox virus genomes. Our assays were capable of detecting the presence of less than 10 plaque-forming units
20 (pfu) of vaccinia virus and could prove to be valuable to those institutions were variola and monkeypox positive controls are unavailable for testing of clinical samples.

The invention comprises a method of detecting orthopoxvirus within a sample comprising providing primers capable of amplifying a region of the orthopoxvirus



genome within the HA gene including restriction enzyme sites for Sau3AI and SpeI and/or primers capable of amplifying a region of the orthopoxvirus genome within the crmB gene including restriction enzyme sites for DraI, Alw44I, SspI and HpaI, as shown in Figure 1. As will be appreciated by one of skill in the art, a wide variety of primers spanning this region and capable of amplifying genomic DNA from at least one of the orthopoxviruses may be used. The sample and the primers are then incubated under conditions suitable for nucleic acid amplification as discussed below. The resulting amplicon(s) are then digested with at least the respective restriction enzymes listed above and the digestion of the amplicon(s) is determined, for example, visually, by DNA electrophoresis, although other suitable methods known in the art for detecting restriction enzyme cleavage, for example, absence of fluorescence quenching may also be used. As shown in Figure 1, the restriction enzyme digestion pattern can then be used to identify the orthopoxvirus, if present within the sample.

In another embodiment, the primer pairs comprise 12 or more consecutive nucleotides of ATGCCGGTACTTATGTATGTGC (nucleotides 151327-151347, SPOXHA5, SEQ ID NO: 1) and 12 or more consecutive nucleotides of TCTTGTCTGTTGTGGATTCT (nucleotides 151656-151637, SPOXHA3, SEQ ID NO: 2) for amplification of the region of the HA gene; and 12 or more consecutive nucleotides of TACCGGTCTCAGCGAATC (nucleotides 183227-183244, SPOXcrmB5, SEQ ID NO: 3) and 12 or more consecutive nucleotides of ACCGTCTCCGAATGCGGCAT (nucleotides 183493-183474, SPOXcrmB3, SEQ ID NO: 4) for amplification of the region of the crmB gene.

In other embodiments, the primer pairs may comprise 13, 14, 15 or more

consecutive nucleotides of ATGCCGGTACTTATGTATGTGC (SPOXHA5, SEQ ID NO: 1) and 13, 14, 15 or more consecutive nucleotides of TCTTGTCTGTTGTGGATTCT (SPOXHA3, SEQ ID NO: 2) for amplification of the region of the HA gene; and/or 13, 14, 15 or more consecutive nucleotides of TACCGGTCTCAGCGAATC (SPOXcrmB5, SEQ ID NO: 3) and 13, 14, 15 or more consecutive nucleotides of ACCGTCTCCGAATGCGGCAT (SPOXcrmB3, SEQ ID NO: 4) for amplification of the region of the crmB gene.

In another embodiment, the primer pairs consist essentially of 12 or more consecutive nucleotides of ATGCCGGTACTTATGTATGTGC (SPOXHA5, SEQ ID NO: 1) and 12 or more consecutive nucleotides of TCTTGTCTGTTGTGGATTCT (SPOXHA3, SEQ ID NO: 2) for amplification of the region of the HA gene; and 12 or more consecutive nucleotides of TACCGGTCTCAGCGAATC (SPOXcrmB5, SEQ ID NO: 3) and 12 or more consecutive nucleotides of ACCGTCTCCGAATGCGGCAT (SPOXcrmB3, SEQ ID NO: 4) for amplification of the region of the crmB gene.

In other embodiments, the primer pairs consist essentially of 13, 14, 15 or more consecutive nucleotides of ATGCCGGTACTTATGTATGTGC (SPOXHA5, SEQ ID NO: 1) and 13, 14, 15 or more consecutive nucleotides of TCTTGTCTGTTGTGGATTCT (SPOXHA3, SEQ ID NO: 2) for amplification of the region of the HA gene; and/or 13, 14, 15 or more consecutive nucleotides of TACCGGTCTCAGCGAATC (SPOXcrmB5, SEQ ID NO: 3) and 13, 14, 15 or more consecutive nucleotides of ACCGTCTCCGAATGCGGCAT (SPOXcrmB3, SEQ ID NO: 4) for amplification of the region of the crmB gene.

As will be known to one of skill in the art, DNA amplification involves allowing

5 which are incorporated herein by reference.

10 denaturation , hybridization and extension is repeated as many times as necessary to
obtain the desired amount of amplified nucleic acid.

15 to catalyze template-dependent DNA synthesis. For example, if the template is RNA,
a suitable polymerizing agent to convert RNA to cDNA is reverse transcriptase, such
as avian myeloblastosis virus RT or Murine Moloney Leukemia Virus RT. If the
template is DNA, suitable polymerases include for example *E. coli* DNA polymerase I,
the Klenow fragment of DNA polymerase I, T₄ DNA polymerase, Hot Tub® and Taq
20 polymerase.

Thus, in one embodiment, primer pairs corresponding to SPOXHA5 and SPOXHA3 and/or SPOXcrmB5 and SPOXcrmB3 are mixed with a sample from a patient suspected of or at risk of orthopoxvirus infection. It is of note that these

primers are described herein for illustrative purposes and other suitable primer pairs as discussed above may also be used. Reagents needed for nucleic acid amplification, discussed above, are added to the sample and the sample is incubated under conditions suitable for nucleic acid amplification, for example, an initial
5 inactivation at 95°C, 40 cycles of 45 seconds at 95°C, 45 seconds at 50°C and 90 seconds at 72°C, followed by a final extension at 72°C for 5 minutes, although other suitable incubation temperatures and times may also be used. If orthopoxvirus DNA is present within the sample, an amplicon will have been generated as a result of nucleic acid amplification. In some embodiments, as discussed above, positive controls may
10 be added or incubated as controls for nucleic acid amplification and restriction enzyme digestion, as discussed below. Restriction enzymes, selected from the group consisting of Sau 3AI, Spe I, Dra I, Alw 44I, Ssp I, Hpa I and combinations thereof are added and the resulting mixture is incubated under conditions suitable for restriction enzyme digestion. Digestion of the amplicon is then confirmed by any of a number of
15 means known in the art, for example, by gel electrophoresis as shown in Figure 2. Specifically, as shown in Figures 1 and 2, digestion of the HA amplicon with Sau 3AI and Spe I shows a specific banding pattern for variola, monkeypox, camelpox and vaccinia/cowpox. Similarly, digestion of the crmB amplicon with Dra I, Alw 44I, Ssp I and Hpa I results in a specific banding pattern for variola, monkeypox, camelpox,
20 vaccinia and cowpox, allowing these viruses to be clearly distinguished from one another.

Specifically, referring to Figures 1 and 2, if the sample tested contains Variola (Genbank Accession L22579), Camelpox (Genbank Accession AF438165), Vaccinia

(Genbank Accession U94848) or Monkeypox DNA (Genbank Accession AF380138), incubation with SPOXHA5/SPOXHA3 and/or SPOXCRMB5/SPOXCRMB3 will result in the product of amplicon(s).

If the Orthopoxvirus DNA is Variola, digestion of the HA amplicon with Sau 3AI
5 (at nucleotide 151462) and Spe I (at nucleotides 151483 and 151600) will produce restriction fragments of approximately 135 nts, 117 nts, 57 nts and 20 nts. Digestion of the crmB amplicon with Dra I (at nucleotide 183398), Alw 44I, Ssp I and Hpa I will produce two bands of approximately 168 nts and 98 nts.

If the Orthopoxvirus DNA is monkeypox, digestion of the HA amplicon with Sau
10 3AI (at nucleotide 159041) and Spe I (at nucleotide 159173) will produce restriction fragments of approximately 134 nts, 132 nts and 57 nts. Digestion of the crmB amplicon with Dra I, Alw 44I, Ssp I and Hpa I (at nucleotide 194885) will produce two bands of approximately 216 nts and 47 nts.

If the Orthopoxvirus DNA is camelpox, digestion of the HA amplicon with Sau
15 3AI (at nucleotides 163771 and 163884) and Spe I (at nucleotide 163909) will produce restriction fragments of approximately 135 nts, 113 nts, 57 nts and 24 nts. Digestion of the crmB amplicon with Dra I, Alw 44I, Ssp I (at nucleotide 202188) and Hpa I will produce two bands of approximately 147 nts and 119 nts.


If the Orthopoxvirus DNA is vaccinia, digestion of the HA amplicon with Sau
20 3AI (at nucleotides 149861 and 149953) and Spe I (at nucleotides 149861 and 149978) will produce restriction fragments of approximately 135 nts, 93 nts, 57 nts, 24 nts and 20 nts. Digestion of the crmB amplicon with Dra I, Alw 44I (at nucleotide 170867), Ssp I and Hpa I (at nucleotide 170923) will produce three bands of

approximately 117-139 nts, 56 nts and 46 nts.

It is of note that the above-described primers are capable of binding to nucleic acid of Variola, Camelpox, Vaccinia and Monkeypox. It is of note that other suitable primers capable of amplifying the above-described genomic regions containing the
5 restriction enzyme sites from one or more of the above Orthopoxviruses may also be used for identifying a specific Orthopoxvirus and are within the scope of the invention.

The kits of the invention comprise at least one pair of primers, comprising 12 or more consecutive nucleotides of ATGCCGGTACTTATGTATGTGC (SPOXHA5, SEQ ID NO: 1) and 12 or more consecutive nucleotides of TCTTGTCTGTTGTGGATTCT
10 (SPOXHA3, SEQ ID NO: 2) for amplification of the region of the HA gene; and/or 12 or more consecutive nucleotides of TACCGGTCTCAGCGAATC (SPOXcrmB5, SEQ ID NO: 3) and 12 or more consecutive nucleotides of ACCGTCTCCGAATGCGGCAT (SPOXcrmB3, SEQ ID NO: 4) for amplification of the region of the crmB gene and a set of instructions, generally written instructions although electronic storage media
15 (e.g., magnetic diskette or optical disk) containing instructions are also acceptable, relating to the use of the primers, hybridization conditions and the like. The kit may also include reagents for nucleic acid amplification, positive controls and suitable restriction enzymes as discussed herein.

We developed diagnostic PCR-RFLP assays to detect and distinguish between
20 variola and monkeypox, whose DNA is generally unavailable, and two other species of Orthopoxvirus, vaccinia and camelpox (Gubser, C. and Smith, G.L. J of Gen. Virol. 83,855-872(2002)). New primer sets for the haemagglutinin (HA) and the cytokine response modifier B (crmB) genes (Ropp, S.L. et al J. Clin. Micro. 33, 2069-2076



(1995); Loparev, V.N. et al J.Clin. Micro. **39**, 94-100 (2001)) were selected by aligning sequences entered into GenBank for all isolates of variola, monkeypox, camelpox, cowpox and vaccinia viruses. Amplicons of approximately 300 base pairs were identified which contained wholly conserved restriction enzyme sites to allow for

5 RFLP analysis (fig.1). Relative sensitivity of the primer sets was determined using a serial dilution of DNA extracted from a vaccinia virus stock (VVT7 at 1×10^8 pfu/ml). Both primer sets were able to detect viral DNA equivalent to 7 pfu per reaction, a level, that is 10-100 fold more sensitive than the previously described sets (Ropp, S.L. et al J. Clin. Micro. **33**, 2069-2076 (1995); Loparev, V.N. et al J.Clin. Micro. **39**,

10 94-100 (2001)).


To ensure our assay would be sufficiently robust for clinical samples regardless of sampling methods or shipping conditions, dilutions of vaccinia virus were sampled using cotton swabs and stored as dry swabs (CDC Guide D. Specimen collection guidelines.), or in 0.5ml viral transport media for 18 hours at 4°C. Additional swabs

15 were also sampled using a commercially available culture system and stored for 18 hours at 4°C, 30°C or -20°C to survey the effect of temperature of storage on sensitivity. DNA was extracted from each swab and assayed for the presence of both HA and crmB sequences. Under all regimes tested, no loss of sensitivity was detected.

20 In addition, we generated artificial templates to produce amplicons corresponding to HA and crmB sequences from variola and monkeypox viruses, to be used as positive controls in RFLP analysis. As a test of our assay, vaccinia strain MVA T7, was used as an "Orthopoxvirus clinical sample" (test sample). DNA was

extracted and PCR reactions for HA and crmB sequences were performed in parallel with reactions using vaccinia and camelpox genomic DNA and the cloned synthetic templates produced for both variola and monkeypox HA and crmB sequences. Appropriately sized amplicons were produced in both the HA and crmB reactions
5 indicating the test sample was positive for Orthopoxvirus DNA (fig 2). Aliquots of all amplicons produced were digested with restriction enzymes along with the appropriate positive controls to identify the species of test sample (fig. 2). Amplicons were directly sequenced to confirm identity.

While the preferred embodiments of the invention have been described above,
10 it will be recognized and understood that various modifications may be made therein, and the appended claims are intended to cover all such modifications which may fall within the spirit and scope of the invention.



CLAIMS

1. A method of detecting and identifying an orthopoxvirus within a sample comprising:

5 adding to the sample reagents for nucleic acid amplification and at least one pair of primers capable of amplifying at least one region of the orthopoxvirus genome, said region of the orthopoxvirus genome selected from the group consisting of HA and crmB;

incubating the sample under conditions suitable for nucleic acid amplification thereby producing an amplicon if the sample contains orthopoxvirus;

10 adding at least one restriction enzyme selected from the group consisting of: Sau 3AI, Spe I, Dra I, Hpa I, Ssp I, Alw 44I and combinations thereof; and

determining if restriction enzyme digestion of an amplicon has occurred.

2. The method according to claim 1 wherein restriction enzyme digestion of an amplicon is determined by gel electrophoresis.

15 3. The method according to claim 1 wherein the pair of primers comprise 12 or more consecutive nucleotides of ATGCCGGTACTTATGTATGTGC (SPOXHA5, SEQ ID NO: 1) and 12 or more consecutive nucleotides of TCTTGTCTGTTGTGGATTCT (SPOXHA3, SEQ ID NO: 2).

4. The method according to claim 1 wherein the pair of primers comprise 20 12 or more consecutive nucleotides of TACCGGTCTCAGCGAATC (SPOXcrmB5, SEQ ID NO: 3) and 12 or more consecutive nucleotides of ACCGTCTCCGAATGCGGCAT (SPOXcrmB3, SEQ ID NO: 4).

5. A pair of primers for detecting orthopoxvirus in a sample comprising 12

or more consecutive nucleotides of ATGCCGGTACTTATGTATGTGC (SPOXHA5, SEQ ID NO: 1) and 12 or more consecutive nucleotides of TCTTGTCTGTTGTGGATTCT (SPOXHA3, SEQ ID NO: 2) or 12 or more consecutive nucleotides of TACCGGTCTCAGCGAATC (SPOXcrmB5, SEQ ID NO: 3) and 12 or
5 more consecutive nucleotides of ACCGTCTCCGAATGCGGCAT (SPOXcrmB3, SEQ ID NO: 4).

6. A kit for detecting and identifying orthopoxvirus comprising:

at least one pair of primers selected from the group consisting of 12 or more consecutive nucleotides of ATGCCGGTACTTATGTATGTGC (SPOXHA5, SEQ ID
10 NO: 1) and 12 or more consecutive nucleotides of TCTTGTCTGTTGTGGATTCT (SPOXHA3, SEQ ID NO: 2); and 12 or more consecutive nucleotides of TACCGGTCTCAGCGAATC (SPOXcrmB5, SEQ ID NO: 3) and 12 or more consecutive nucleotides of ACCGTCTCCGAATGCGGCAT (SPOXcrmB3, SEQ ID NO: 4).

15 7. The kit according to claim 6 including at least one restriction enzyme selected from the group consisting of: Sau 3AI, Spe I, Dra I, Hpa I, Ssp I, Alw 44I and combinations thereof.

8. The kit according to claim 6 including at least one positive control DNA template capable of binding to both primers of at least one primer pair.

ABSTRACT

We have developed two new PCR based assays to detect Orthopoxvirus DNA which when tested under simulated clinical conditions, have proven to be very sensitive, rapid and robust. Importantly, we were able to construct artificial templates
5 for use as positive controls in the RFLP analysis necessary to differentiate variola and monkeypox viruses from the Orthopoxvirus species that normally cause relatively insignificant disease in man. Our assays will provide a lab that has already undertaken the isolation of DNA from a patient sample in biocontainment, a sensitive and controlled assay for the detection and differentiation of human-tropic orthopox
10 viruses.




Figure 1

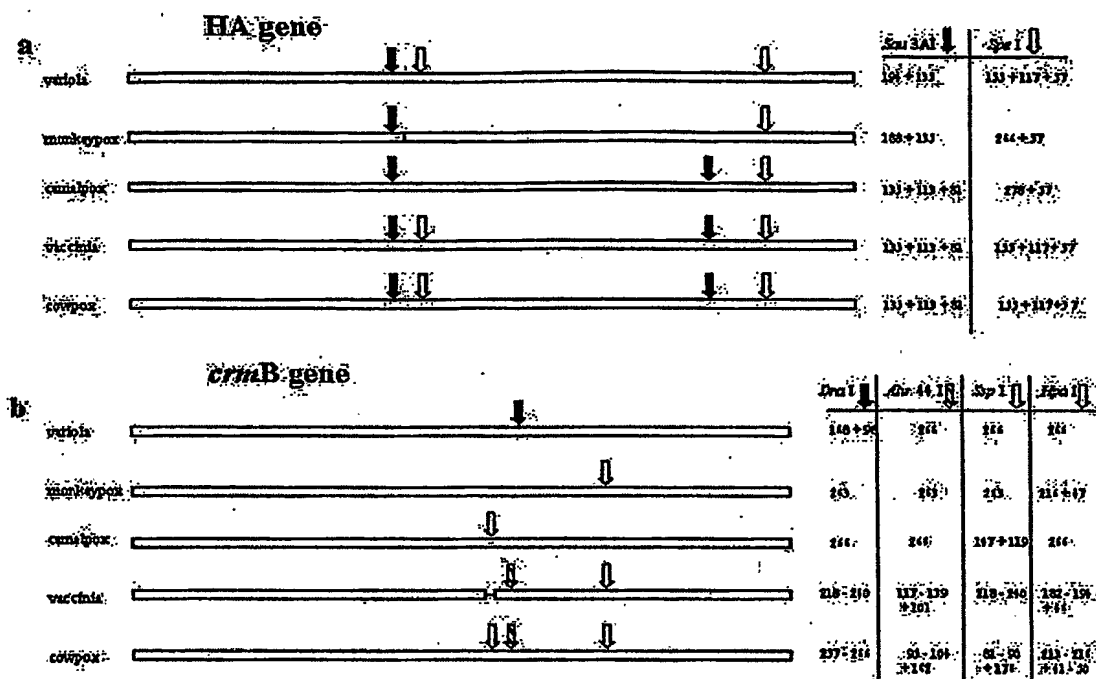
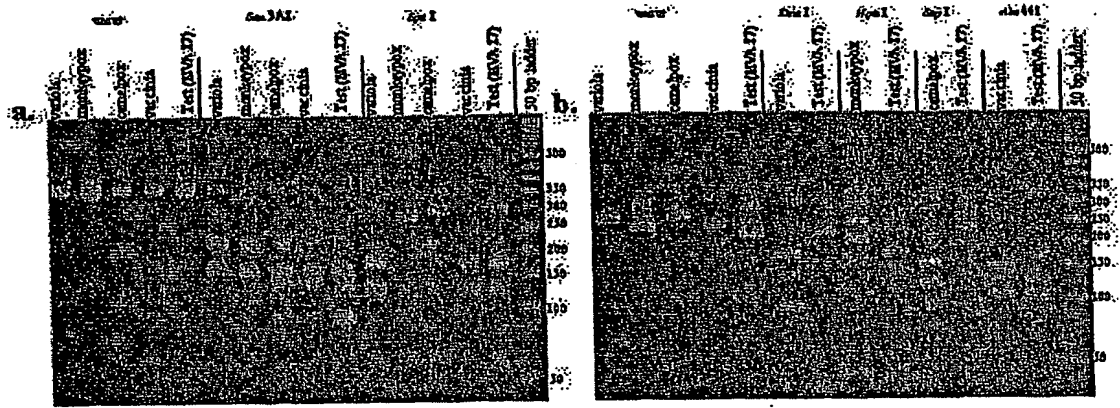


Figure 2



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